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(54) Title: DIFFERENTIAL DIAGNOSIS OF DISORDERS OF IRON METABOLISM BY MEANS OF THREE INDEPENDENT PARAMETERS AND RECOMMENDATIONS FOR THE TREATMENT OF THESE DISORDERS OF IRON METABOLISM

(57) Abstract: The invention concerns a method for detecting disorders of iron metabolism and in particular the differential diagnosis of disorders of iron metabolism by means of three independent parameters. The differential diagnosis can be used to classify disorders of iron metabolism and to recommend the required treatment and to monitor the progress and response to treatment.

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**Differential diagnosis of disorders of iron metabolism by means of three independent parameters and recommendations for the treatment of these disorders of iron metabolism**

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**Description**

The invention concerns a method for detecting disorders of iron metabolism and in particular the differential diagnosis of disorders of iron metabolism by means of three independent parameters. The differential diagnosis can be used to classify disorders of iron metabolism and to recommend the required treatment and to monitor the progress and response to treatment.

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Iron as a component of haemoglobin and the cell haemins is one of the most important biocatalysts in the human organism. Disorders of iron metabolism and in particular iron deficiency and perturbations of iron distribution and utilization in chronic general illnesses are among the most frequently overlooked or misinterpreted diseases. One of the main reasons for this is that the determination of transport iron in the serum or plasma which is used in conventional diagnostics does not allow a representative estimation of the total body iron stores due to short-term variations.

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The ability to precisely determine the iron storage protein ferritin in plasma provided a method for determining the total body iron stores and thus allowed a more rapid and reliable diagnosis especially of iron deficiency states. Ferritin is an indicator of the amount of storage iron. The soluble transferrin receptor (sTfR) indicates the iron requirements of the cell and erythropoiesis activity. The sTfR/log ferritin index is a measure of the depletion of the iron stores and of the functional iron compartments. In chronic inflammatory diseases such as in infections and especially tumour

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diseases, iron is redistributed with a relative overload of the iron stores accompanied by a relative deficiency of iron supply to the erythropoietic cells.

5 Due to the very limited capacity to absorb iron, the iron requirements can only be met by recycling functional iron. It is stored in the form of ferritin and haemosiderin. Each cell is able to take up a surfeit of iron by synthesizing ferritin and the basic mechanisms for this are identical in all types of cells. The transferrin-iron<sup>3+</sup> complex is bound to the transferrin 10 receptor of the cell membrane. The uptake of iron can be regulated by the transferrin receptor expression. In addition iron induces the synthesis of apoferritin. Hence in the majority of metabolic situations a representative proportion of the synthesized ferritin is released into the blood plasma.

15 However, even if the above-mentioned parameters are employed, it is not in practice possible or very difficult to routinely determine and differentiate between various iron states.

20 Therefore an object of the present invention was to provide a method which enables the reliable detection of disorders of iron metabolism in a simple manner.

25 This object is achieved according to the invention by a method for determining the iron status and in particular for detecting disorders of iron metabolism comprising the determination of

- (i) a parameter which allows a determination of the total body iron stores,
- (ii) a parameter which allows a determination of the erythropoietic maturation process and/or its activity and
- 30 (iii) a parameter which allows a determination of unspecific disorders of iron metabolism.

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Hence the invention concerns the differential diagnosis of disorders of iron metabolism by means of three independent parameters.

The determination of the total body iron stores can for example be carried out by measuring the parameters erythrocyte ferritin, zinc protoporphyrin, haemoglobin, myoglobin, transferrin and transferrin saturation, ferritin, haemosiderin or/and the enzymes catalase, peroxidase or/and cytochrome. A determination of the concentration or activities of these parameters enables a determination of the total body iron stores which is determined as parameter (i) of the method according to the invention. Ferritin or transferrin and particularly preferably ferritin is used as the parameter.

The erythropoietic maturation process and/or the erythropoietic activity can for example be ascertained or determined using erythrocyte indices, reticulocyte indices, FS-e (forward scatter erythrocytes) and/or the soluble transferrin receptor (sTfR). The amount or concentration of soluble transferrin receptor (sTfR) is particularly preferably determined as parameter (ii) in the method according to the invention and used as a parameter for the erythropoietic maturation process or its activity.

Biochemical parameters as well as haematological parameters can be used as a parameter for determining unspecific disorders of iron metabolism. Acute phase proteins and regulators of acute phase protein synthesis are preferably used as biochemical parameters whereas disorders of reticulocyte synthesis are preferably used as haematological parameters. Examples of acute phase proteins whose amount or concentration is determined in order to determine unspecific disorders of iron metabolism comprise C-reactive protein (CRP), serum amyloid A (SAA),  $\alpha_1$ -anti-chymotrypsin, acidic  $\alpha_1$ -glycoprotein,  $\alpha_1$ -antitrypsin, haptoglobin, fibrinogen, complement component C3, complement component C4 or/and coeruloplasmin. Examples of regulators of acute phase protein synthesis are interleukin 6 (IL-6), leukaemia inhibiting factor (LIF), oncostatin M,

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interleukin 11 (IL-11), ciliary neurotropic factor (CNTF), interleukin 1 $\alpha$  (IL-1 $\alpha$ ), interleukin 1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor- $\alpha$  (TNF $\alpha$ ), tumour necrosis factor- $\beta$  (TNF $\beta$ ), insulin, fibroblast growth factor (FGF), hepatocyte growth factor, transgrowth factor  $\beta$  (TGF $\beta$ ) or/and interferon.

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Disorders of reticulocyte synthesis such as CH<sub>2</sub>, reticulocyte count, Hb content of reticulocytes (CHr), IRF (immature reticulocyte fraction) new RBC and reticulocyte fluorescence parameters and/or FS-r (forward scatter reticulocytes) are haematological parameters that can be used in particular as parameter (iii) of the method according to the invention. CRP, SAA or/and CHr are preferred.

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According to the invention it was surprisingly found that rapid and reliable information on the iron status of patients can be obtained by combining three independent parameters. In particular it was found that biochemical or haematological markers and in particular inflammatory markers which are unspecific as such, can be used in an appropriate combination with other parameters to determine the iron status.

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In particular the method according to the invention allows a classification of the iron status and in particular of disorders of iron metabolism.

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The combination of three independent parameters enables a routine differentiation between normal iron status, iron deficiency, iron distribution disorders and/or iron overloading. In particular the method according to the invention allows a differentiation between normal iron status and iron overloading. In addition it allows a differentiation between the status of iron deficiency and iron distribution disorders. Perturbations of iron distribution can lead to chronic diseases such as rheumatism, asthma or tumours and hence an early detection of iron distribution disorders is of particular importance.

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In a particularly preferred embodiment the iron status determined by the method according to the invention is classified in one of the following groups:

- 5 (A) iron distribution disorder or/and iron utilization disorder with acute phase reaction,
- (B) iron overloading,
- (C) normal iron status, and
- (D) deficiency of storage iron.

10 The evaluation of the determined parameters can be preferably assisted by a computer for example by use of an anaemia program. Furthermore the determined measurements are preferably represented graphically in the form of diagrams in order to easily assign the measuring ranges to the various iron states. For example parameter (iii) can be plotted on the 15 ordinate and the ratio of parameter (ii) to parameter (i) can be plotted on the abscissa. This results in various measuring ranges (fields in the diagram) for the various iron states and iron overloading can be distinguished from a normal iron status, and a normal iron status can be distinguished from iron deficiency and also from iron distribution 20 disturbances such as tumour anaemia, chronic anaemia, rheumatoid arthritis or renal anaemia.

25 The method according to the invention can also be used to specify in a simple manner the treatment required for the respective patient depending on the determined iron status. Thus for example erythropoietin (EPO) therapy is indicated for a classification in group (A), blood letting is indicated for a classification in group (B), no therapy is indicated for a classification in group (C) and iron substitution is indicated when classified in group (D). These therapeutic recommendations are based on the fact 30 that erythropoiesis is mainly regulated by the growth factor EPO and by iron, and the various types of iron metabolism disorders require different treatments that can be determined by the method according to the

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invention. An iron deficiency leads in particular to a deficit in haemoglobin formation, to hypochromic mycrocytes/anulocytes and thus to anaemias which are manifested as iron deficiency and chronic bleeding. Deficiency of erythropoietin (EPO) results in a reduced proliferation and thus to anaemias that manifest themselves as iron distribution disturbances, acute phase conditions, infections, chronic inflammation, tumour anaemias and renal anaemias.

10 In addition to the treatment of disorders of iron metabolism, the method according to the invention also allows observation or/and monitoring of the progress and response to treatment and thus ensures an optimal use of EPO or iron preparations (e.g. oral or parenteral iron preparations) in individual patients.

15 Depending on the selected characteristic values of the above mentioned parameters, the method according to the invention also allows a sex-specific discrimination or differentiation of the individual iron status in which the normal values or cut-off values can then be established for each sex (male or female).

20 Surprisingly, it was found that chronic diseases, even in very early stages, result in a classification in group (A). Thus, chronic diseases and chronic inflammatory diseases can be diagnosed with the method according to the invention. In particular, diseases such as renal insufficiency, malignancies, 25 rheumatoid arthritis, diabetes, heart failure, cardiovascular diseases, thrombosis, neurogenerative diseases or impaired pregnancies can be identified, and respective treatments can be indicated by the present invention.

30 Group (B) indicating iron overloading includes haemochromatosis such as sickle cell anemia or HFE gene modifications.

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The invention is elucidated in the following on the basis of particularly preferred embodiments; however, it should be noted that the inventive procedure is not limited to the parameters mentioned as examples.

5 In a first preferred embodiment sTfR is determined as parameter (ii). Surprisingly it was found that the soluble transferrin receptor (sTfR) is a parameter for the following three types of iron status:

(a) haemoglobin synthesis rate,  
(b) repletion status of the iron stores (ferritin) and  
10 (c) non-ferritin iron deposition (disturbance in distribution, iron deposition).

In addition it is preferred that the ferritin content is determined as parameter (i). A combination of sTfR and ferritin yields information on the 15 depletion of iron stores, haemoglobin synthesis and iron deposition as shown in figure 1.

These two parameters for determining the iron status i.e. sTfR and ferritin can now be combined in a preferred embodiment of the method according 20 to the invention with a further biochemical marker or a haematological marker.

The inflammation marker CRP or the marker SAA and most preferably the marker CRP is used as the biochemical marker.

25 This combination can serve in particular as diagnostic markers for chronic anaemias (ACD) in rheumatic diseases.

In order to efficiently differentiate between the anaemias, the classification 30 is carried out by calculating the ratio of sTfR/log ferritin. It is standardized on the basis of the CRP value. For the graphic representation the ratio of sTfR/log ferritin is plotted on the X axis and the CRP value is plotted on the

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Y axis. This results in the following classification into the various types of iron status shown in table 1:

5 Table 1: Differentiation and treatment recommendations for various anaemias using sTfR, ferritin and CRP values

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Quadrant*	Ferritin [ $\mu$ g/L]	sTfR [mg/L]	<u>sTfR</u> log ferritin	CRP [mg/L]	Comments
A	> 30 ♂ > 15 ♀	high↑ (measure of erythropoietic activity)	< 3.4 ♂ < 3.7 ♀	> 5 > 5	disturbances of iron distribution disturbances of iron utilization with acute phase reaction (EPO therapy)
B	> 400 ♂ > 150 ♀	< 5 > 4.4	< 0.9 ♂ < 0.9 ♀	< 5 < 5	iron overloading (blood letting therapy)
C	30-400 ♂ 15-150 ♀	> 5 > 4.4	< 3.4 ♂ < 3.7 ♀	< 5 < 5	normal iron status, no acute phase reaction
D	< 30 ♂ < 15 ♀	> 5 > 4.4	> 3.4 ♂ > 3.7 ♀	< 5 < 5	deficiency of stored iron, no acute phase reaction (iron substitution)
	< 30 ♂ < 15 ♀	very high↑↑ (measure of iron requirements of the cells)	> 3.4 ♂ > 3.7 ♀	> 5	deficiency of stored iron with acute phase reaction (iron substitution)

\* see Fig.2

25 The cut-off values shown in table 1 are derived from the reference ranges for women (premenopausal) for sTfR of 1.9 to 4.4 mg/l, ferritin of 15 to 150  $\mu$ g/l and CRP of < 5 mg/l and for men for sTfR of 2.2 to 5.0 mg/l, ferritin of 30 to 400  $\mu$ g/l and CRP of < 5 mg/l. When this is represented graphically results in four quadrants which are defined by the cut-off values for CRP of 5 mg/l and for the ratios sTfR/log ferritin of 3.4 (men) and 3.7 (women) and 0.9. This enables anaemias which are caused by perturbations of iron distribution (A), iron deficiency (D) and iron overloading (B) to be distinguished from the normal iron status (C).

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In a particularly advantageous embodiment of the invention the differential diagnosis of the important disorders of iron metabolism is assisted by a software program which enables a mathematical linkage of the three above-mentioned independent parameters. The following independent 5 parameters are preferably used:

- (i) ferritin as a parameter that allows an estimate of the actual body iron stores (depot iron),
- (ii) sTfR as a parameter which allows an estimation of the erythropoietic activity (functional iron) and
- 10 (iii) CRP as a parameter for the diagnosis of unspecific disorders of iron metabolism

which are caused for example by inflammatory processes.

15 In this manner the method according to the invention enables disorders of iron metabolism to be described by using the iron storage protein ferritin and the soluble transferrin receptor as an indicator for the iron requirements of the cells. In addition the determination of the soluble transferrin receptor enables an estimate of the erythropoietic activity. CRP acts as an indicator of a persistent acute phase reaction. The correlation 20 between CRP and the ratio of sTfR/log ferritin allows an efficient differential diagnosis of anaemias such as iron deficiency, iron distribution disorders and iron overloading from normal iron status. The differential diagnosis can be further simplified for the user by a computer-aided 25 evaluation program.

A latex-enhanced immunoturbidimetric assay can for example be used to determine the soluble transferrin receptor for use in a method in combination with the determination of ferritin and CRP. The values for sTfR 30 stated herein in connection with methods using sTfR, ferritin and CRP refer to values measured with latex-enhanced immunoturbidimetric assays. The latex-enhanced immunoturbidimetric assay have an adequately sensitive

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measuring accuracy for detecting the relatively low concentrations of soluble transferrin receptor in the blood plasma (< 10 mg/l, or < 100 nmol/l). Since international reference methods and reference preparations are not yet available for sTfR, reference intervals on the COBAS INTEGRA® 5 and Roche/Hitachi were determined for the test described herein and the sTfR reference range was 2.2 to 5.0 (2.5 to 97.5 percentile) for men and 1.9 to 4.4 for women.

According to the invention the cut-off value for sTfR/log ferritin which 10 discriminates between the iron status of iron overloading and normal iron status is 0.7 to 1.4, in particular 0.8 to 1.0 and most preferably 0.9. The cut-off value with which iron deficiency can be distinguished from iron distribution disorders and normal iron status is preferably 3.0 to 4.0, more preferably 3.4 to 3.7 and most preferably at about 3.4 for men and at 15 about 3.7 for women. Calibration to determine these values was made as described by S.Kolbe-Busch et al., Clin.Chem.Lab.Med.40(5) (2002), 529-536. sTfR from placenta was used as standard thereby. The cut-off value for CRP above which an acute phase reaction is defined, is preferably at about 1 to 10 mg/l, more preferably at 4 to 6 mg/l and in particular at 20 about 5 mg/l.

In a further most preferred embodiment a haematological parameter is determined as parameter (iii) and in particular the proportion of hypochromic red blood cells (HRC%) or the haemoglobin content of 25 reticulocytes (CHr). It was surprisingly found that these parameters are new indicators for functional iron deficiency. These parameters can be used in addition to biochemical markers such as ferritin, transferrin saturation (TfS) and transferrin receptor (TfR) to identify an iron deficiency (ID).

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The haematological parameters show rapidly and directly any change in erythropoietic activities.

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Non-anaemic patients without APR (acute phase reaction) have a CHr of  $\geq$  28 pg and HCR of  $\leq$  5 %. Patients with a CHr of  $<$  28 pg or a HCR of  $>$  5 % were classified as functionally iron deficient. Serum ferritin, TfS, TfR and the calculated parameters TfR-F index (ratio TfR/log ferritin) and Tf-Tf-R product enable a reliable diagnosis of iron deficiency in comparison with HCR % and CHr in patients without APR. In the case of anaemias without APR which are often observed in infections, inflammation or tumours, the diagnostic effectiveness of the said biochemical markers ferritin and transferrin receptor is often inadequate. A combination of these biochemical markers with haematological markers such as CHr considerably improves the results. When CHr is plotted against the TfR-F index or against the Tf-TfR product, it is possible to classify anaemias in patients with and without APR inter alia into the following categories: no functional iron deficiency, functional iron deficiency combined with depleted iron stores and functional iron deficiency combined with replete iron stores.

This embodiment of the invention enables an identification of iron deficiency and a distinction of iron deficiency from other disorders of iron metabolism, in particular so-called anaemias, from chronic diseases (ACD) which accompany infections, inflammation or tumours. ACD is characterized by an inadequate erythropoietin production, inhibition of the proliferation of erythrocyte precursor cells in the bone marrow and disturbances of iron utilization. As in iron deficiency anaemia (IDA), functional iron deficiency in ACD is one of the main distinguishing factors from erythropoiesis. It is defined as an imbalance between iron requirements in the erythroid bone marrow and iron supply which is not sufficient to ensure a normal haemoglobinization of red blood cells. This results in a reduced haemoglobin concentration in reticulocytes and erythrocytes. In IDA the iron supply depends on the content of the iron stores, and in the case of ACD on the rate of its mobilization. In ACD a functional iron deficiency can occur even in the presence of large iron stores if the iron release is impaired.

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The diagnosis of a functional iron deficiency is important for the correct treatment of the patients. However, in practice it is often only possible to classify the patients as iron deficient, non-iron deficient or potentially iron-deficient. The third group of patients which are typically those with an 5 acute phase reaction (APR) or a cancer related anaemia (CRA) have previously required an examination of their bone marrow in order to determine the type of disease.

Usually biochemical markers of iron metabolism are used such as serum or 10 plasma iron, transferrin, % transferrin saturation (TfS), ferritin and serum-circulating transferrin receptor (TfR). The diagnosis of IDA is based on the presence of anaemia and morphological features of erythrocytes (hyperchromia, mycrocytosis) in conjunction with a low serum ferritin and a reduced transferrin saturation. The diagnosis of ID in conjunction with 15 normal serum ferritin contents may, however, be difficult in the case of ACD. Ferritin is an acute phase reactant, transferrin is a negative acute phase reactant and the concentration of both proteins is influenced by various conditions. An increase in TfR which is a useful indicator for iron deficiency, can also occur in patients with an increase in the number of red 20 precursor cells in the bone marrow. Due to these difficulties it is necessary to provide clinical laboratory tests which measure the functional availability of iron for haemoglobin synthesis especially in the red blood cells and their precursors.

25 A marker which can be used to assess the functional iron status, is the measurement of the proportion of hypochromic red cells (HRC %). Due to the life time of erythrocytes of about 120 days, HCR % integrates information over a long period and is thus a late indicator for iron-limited erythropoiesis. A value for HCR of < 10 % in conjunction with low serum 30 ferritin indicates that the iron supply for erythropoiesis is sufficient to enable a normal haemoglobinization of red cells.

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The cellular haemoglobin content of reticulocytes (CHr) is an early marker for functional iron deficiencies since reticulocytes exist in the circulation for only 1 to 2 days. The utility of this index for monitoring the erythropoietic function in order to assess the iron status, to diagnose an iron deficiency and to diagnose and treat various haematological diseases is known.

A combination of the haematological indices HRC % or/and CHr with biochemical markers is described here for the first time.

10 Using the 2.5 and 97.5 percentiles of the control group, the following cut-offs were determined for the present invention: 3 to 7 %, in particular 4 to 6 % and most preferably about 5 % for HCR and 25 to 30 pg, in particular 27 to 29 pg and particularly preferably about 28 pg for CHr. The iron status can preferably be classified using a diagnostic plot in which CHr is plotted against TfR-F or against Tf-Tf-R. In this manner the iron status can be divided into various categories and in particular four categories i.e. normal iron status, iron deficiency (CRP normal), iron deficiency (CRP increased) and iron distribution disorder.

20 In a further preferred embodiment the invention relates to a method for determining the iron status and, in particular, for detecting disorders of iron metabolism comprising the determination of

- (i) a parameter which allows determination of the total body iron stores,
- (ii) a parameter which allows determination of the erythropoietic maturation process and/or its activity,
- (iii) a parameter which allows determination of unspecific disorders of iron metabolism, in particular, a biochemical parameter, and
- (iv) a haematological parameter, in particular, MCH or CHr.

30 In this embodiment group (A) concerning patients who probably have disturbances of iron distribution (acute deficiency of functional iron) can be

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further divided in two groups. In particular, patients having no acute deficit of functional iron can be distinguished from patients actually having functional iron deficiency or disturbance of iron distribution. MCH or CHr can be determined from blood count. MCH is the average hemoglobin content of an erythrocyte cell and is reduced, if an acute deficiency of functional iron and thus a disturbance of iron distribution occurs. Therefore, MCH can be used to distinguish a deficiency of functional iron from other other conditions. 28 pg/cell is to be regarded as a limiting value of MCH and CHr, whereby no acute deficiency of functional iron is the case for values above that value and deficiency of functional iron is diagnosed, if values are lower.

The invention further relates to a test strip for performing the inventive method. Such a test strip comprises means for the determination of

- 15 (i) a parameter which allows determination of the total body iron stores,
- (ii) a parameter which allows determination of the erythropoietic maturation process and/or its activity, and
- (iii) a parameter which allows determination of unspecific disorders of
- 20 iron metabolism.

In a preferred embodiment, for example, CRP will be determined competitively and the other two parameters by using a sandwich assay.

25 The invention is further elucidated by the attached figures and examples.

Figure 1 shows that the soluble transferrin receptor (sTfR) is a parameter for three types of iron status. For (A) (iron distribution disturbance) this means Hb synthesis plus iron deposition, for (B) (iron overloading) Hb synthesis plus iron deposition, for (C) (normal iron status) Hb synthesis and for (D) (iron deficiency) Hb synthesis plus storage iron.

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Figure 2 shows an example of an input for an anaemia program, the classification of the four quadrants A, B, C, D in a diagram of CRP against sTfR/log ferritin, the classification of the squares and the treatment recommended in each case.

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Figure 3 shows the classification used in a combination of haematological and biochemical markers.

10 Figure 4 shows a preferred embodiment according to the invention, wherein group (A) is further divided by determination of a haematological parameter, in particular, of MCH or CChr.

#### Example 1

15 163 patients were examined using the parameters CRP and sTfR/log ferritin and classified according to the results obtained as normal iron status, iron deficiency, iron distribution disturbance or iron overloading. The combined determination of the three parameters sTfR, ferritin and CRP proved to be highly suitable for differential diagnosis.

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#### Example 2

25 373 patients were examined using a combination of haematological parameters and biochemical parameters and classified into four groups. Group N is the control group and contained non-anaemic patients without APR. Group A consists of anaemic patients without APR. Group AA contains anaemic patients with APR in combination with CRA, ACD or an acute infectious or inflammatory disease. The patient group NA contains non-anaemic patients with APR.

30 Ferritin was determined on a Cobascore analyzer from Roche Diagnostics, Mannheim, Germany and the reference range was determined as 20 to 150  $\mu\text{g/l}$  for women and 20 to 350  $\mu\text{g/l}$  for men. TfR was determined in each sample using commercial assays. The analytical principle of the assay

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(Dadebehring, Marburg) is based on microagglutination of latex particles which are coated with a monoclonal anti-TfR antibody. In this manner a latex-enhanced nephelometric test is carried out. The reference range (2.5 to 97.5 percentile) was 0.4 to 1.8 mg/l.

5

TfS was calculated using the formula  $TfS (\%) = Fe (\mu\text{g/l}) \times 7.09/Tf (\text{g/l})$ .

In order to determine disorders of iron metabolism CHr and HRC % were determined as indicators of an iron deficient erythropoiesis as a plot against 10 the TfR-F index. The following results were obtained for the individual patient groups.

N group (non-anaemic group without APR)

15 The control group consisted of 71 patients which were found in quadrant 1 (left top, fig. 3) in the diagnostic blots comprising 4 quadrants.

A group (anaemic group without APR)

20 79 anaemic patients without APR were examined and assigned to quadrant 2 (figure 3).

NA group (non-anaemic group with APR)

25 This group consisted of 80 patients which were classified in quadrant 4 (figure 3).

25

AA group (anaemic group with APR)

This group consisted of 143 patients which were classified in quadrant 3 (figure 3).

Patients with data points in quadrant 1 had a CHr of  $\geq 28 \text{ pg}$ .

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Patients in quadrant 2 are iron-deficient according to the TfR-F index. All patients in this quadrant have a CAA and HRC  $> 5 \%$ . The pattern CHr  $>$

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288 pg, HRC > 5 %, elevated TfR and normal or elevated ferritin indicated that these patients with CRA and APR have a reduced iron supply as indicated by the increase in TfR which, however, was not sufficient to cause a functional iron deficiency.

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Patients with data points in quadrant 3 had the lowest ferritin and highest Tf concentrations. Tf is a negative acute phase reactant and the mean concentration was reduced in patients with an iron replete status in quadrants 1 and 4. In patients of quadrant 3 with haematological and biochemical identified iron deficiency, APR did not, however, cause a decrease in the serum Tf which indicates that the positive stimulus of iron deficiency is larger than the negative stimulus of APR on Tf synthesis.

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The patients with data points in quadrant 4 had a CHr of < 28 pg and a HRC of > 5%.

In summary this means that the allocation of the data points to one of the quadrants 1 to 4 in the diagnostic plot denotes the following for the identification of iron deficiency in the diagram CHr against TfR/log ferritin:

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Quadrant 1: no biochemical or haematologically identified iron deficiency  
Quadrant 2: only biochemically identified iron deficiency  
Quadrant 3: biochemically and haematologically identified iron deficiency  
Quadrant 4: only haematologically identified iron deficiency.

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The patient groups can be subdivided as follows according to the haematological and biochemical results:

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Group N: non-anaemic, no APR; Hb (men)  $\geq$  140 g/l, Hb (women)  $\geq$  123 g/l, CRP  $\leq$  5 mg/l, WBC  $\leq$  10,000  $\mu$ l, ESR (erythrocyte sedimentation rate)  $\leq$  30 mm/h, RDW (red cell distribution width)  $\leq$  15 %;  
Group A: anaemic, no APR; Hb (men) < 140 g/l, Hb (women) < 123 g/l,

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CRP  $\leq$  5 mg/l, WBC  $\leq$  10,000/ $\mu$ l; ESR  $\leq$  30 mm/h;

Group NA: non-anaemic with APR; Hb (men)  $\geq$  140 g/l, Hb (women)  $\geq$  123 g/l, CRP  $>$  5 mg/l or WBC  $>$  10,000/ $\mu$ l or ESR  $>$  30 mm/h or RDW  $>$  15 %;

5 AA: anaemic with APR: Hb (men)  $<$  140 g/l, Hb (women)  $<$  123 g/l, CRP  $>$  5 mg/l or WBC  $>$  10,000/ $\mu$ l or ESR  $>$  30 mm/h.

## Claims

1. Method for determining the iron status and in particular for detecting disorders of iron metabolism comprising the determination of
  - (i) a parameter which allows a determination of the total body iron stores,
  - (ii) a parameter which allows a determination of the erythropoietic maturation process and/or its activity and
  - (iii) a parameter which allows a determination of unspecific disorders of iron metabolism.
2. Method as claimed in claim 1,  
characterized in that  
the parameter (i) is selected from erythrocyte ferritin, zinc protoporphyrin, haemoglobin, myoglobin, transferrin, transferrin saturation, ferritin, haemosiderin, catalase, peroxidase or/and cytochrome.
3. Method as claimed in claim 1 or 2,  
characterized in that  
the parameter (ii) is selected from erythrocyte indices, reticulocyte indices, FS-e (forward scatter erythrocytes) and/or soluble transferrin receptor (sTfR).
4. Method as claimed in one of the previous claims  
characterized in that  
the parameter (iii) is selected from acute phase proteins, in particular C-reactive protein (CRP), serum amyloid A (SAA),  $\alpha$ 1-antichymotrypsin, acidic  $\alpha$ 1-glycoprotein,  $\alpha$ 1-antitrypsin, haptoglobin, fibrinogen, complement component C3, complement component C4 or coeruloplasmin, or/and regulators of acute phase

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protein synthesis in particular interleukin 6 (IL-6), leukemia inhibiting factor (LIF), oncostatin M, interleukin 11 (IL-11), ciliary neurotropic factor (CNTF), interleukin 1 $\alpha$  (IL-1 $\alpha$ ), interleukin 1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor- $\alpha$  (TNF $\alpha$ ), tumour necrosis factor- $\beta$  (TNF $\beta$ ), insulin, fibroblast growth factor (FGF), hepatocyte growth factor, transgrowth factor  $\beta$  (TGF $\beta$ ) or interferon (INF) or/and disorders of reticulocyte synthesis in particular reticulocyte count, Hb content of reticulocytes (CHr), immature reticulocyte fraction (IRF), new red blood cell (RBC) and reticulocyte fluorescence parameters or forward scatter reticulocyte (FS-r).

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 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9225 9230 9235 9240 9245 9250 9255 9260 9265 9270 9275 9280 9285 9290 9295 9300 9305 9310 9315 9320 9325 9330 9335 9340 9345 9350 9355 9360 9365 9370 9375 9380 9385 9390 9395 9400 9405 9410 9415 9420 9425 9430 9435 9440 9445 9450 9455 9460 9465 9470 9475 9480 9485 9490 9495 9500 9505 9510 9515 9520 9525 9530 9535 9540 9545 9550 9555 9560 9565 9570 9575 9580 9585 9590 9595 9600 9605 9610 9615 9620 9625 9630 9635 9640 9645 9650 9655 9660 9665 9670 9675 9680 9685 9690 9695 9700 9705 9710 9715 9720 9725 9730 9735 9740 9745 9750 9755 9760 9765 9770 9775 9780 9785 9790 9795 9800 9805 9810 9815 9820 9825 9830 9835 9840 9845 9850 9855 9860 9865 9870 9875 9880 9885 9890 9895 9900 9905 9910 9915 9920 9925 9930 9935 9940 9945 9950 9955 9960 9965 9970 9975 9980 9985 9990 9995 10000 10005 10010 10015 10020 10025 10030 10035 10040 10045 10050 10055 10060 10065 10070 10075 10080 10085 10090 10095 10100 10105 10110 10115 10120 10125 10130 10135 10140 10145 10150 10155 10160 10165 10170 10175 10180 10185 10190 10195 10200 10205 10210 10215 10220 10225 10230 10235 10240 10245 10250 10255 10260 10265 10270 10275 10280 10285 10290 10295 10300 10305 10310 10315 10320 10325 10330 10335 10340 10345 10350 10355 10360 10365 10370 10375 10380 10385 10390 10395 10400 10405 10410 10415 10420 10425 10430 10435 10440 10445 10450 10455 10460 10465 10470 10475 10480 10485 10490 10495 10500 10505 10510 10515 10520 10525 10530 10535 10540 10545 10550 10555 10560 10565 10570 10575 10580 10585 10590 10595 10600 10605 10610 10615 10620 10625 10630 10635 10640 10645 10650 10655 10660 10665 10670 10675 10680 10685 10690 10695 10700 10705 10710 10715 10720 10725 10730 10735 10740 10745 10750 10755 10760 10765 10770 10775 10780 10785 10790 10795 10800 10805 10810 10815 10820 10825 10830 10835 10840 10845 10850 10855 10860 10865 10870 10875 10880 10885 10890 10895 10900 10905 10910 10915 10920 10925 10930 10935 10940 10945 10950 10955 10960 10965 10970 10975 10980 1098

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9. Method as claimed in claim 7 or 8,  
characterized in that  
the required treatment is recommended on the basis of the  
classification of the disorder of iron metabolism.

5

10 10. Method as claimed in claim 9,  
characterized in that  
an EPO therapy is indicated for classification in group (A), blood  
letting is indicated for a classification in group (B), no therapy is  
indicated for a classification in group (C) and iron substitution is  
indicated when classified in group (D).

15 11. Method as claimed in one of the previous claims,  
characterized in that  
the progress or/and response to treatment is observed or/and  
monitored.

20 12. Method as claimed in one of the previous claims,  
comprising the determination of  
(i) a parameter which allows a determination of the total body iron  
stores,  
(ii) a parameter which allows a determination of the erythropoietic  
maturation process and/or its activity and  
(iii) a parameter which allows a determination of unspecific disorders  
25 of iron metabolism, in particular, a biochemical parameter, and  
(iv) a haematological parameter.

30 13. Test strip comprising means for performing a method according to  
any of the preceding claims.

Fig. 1/4

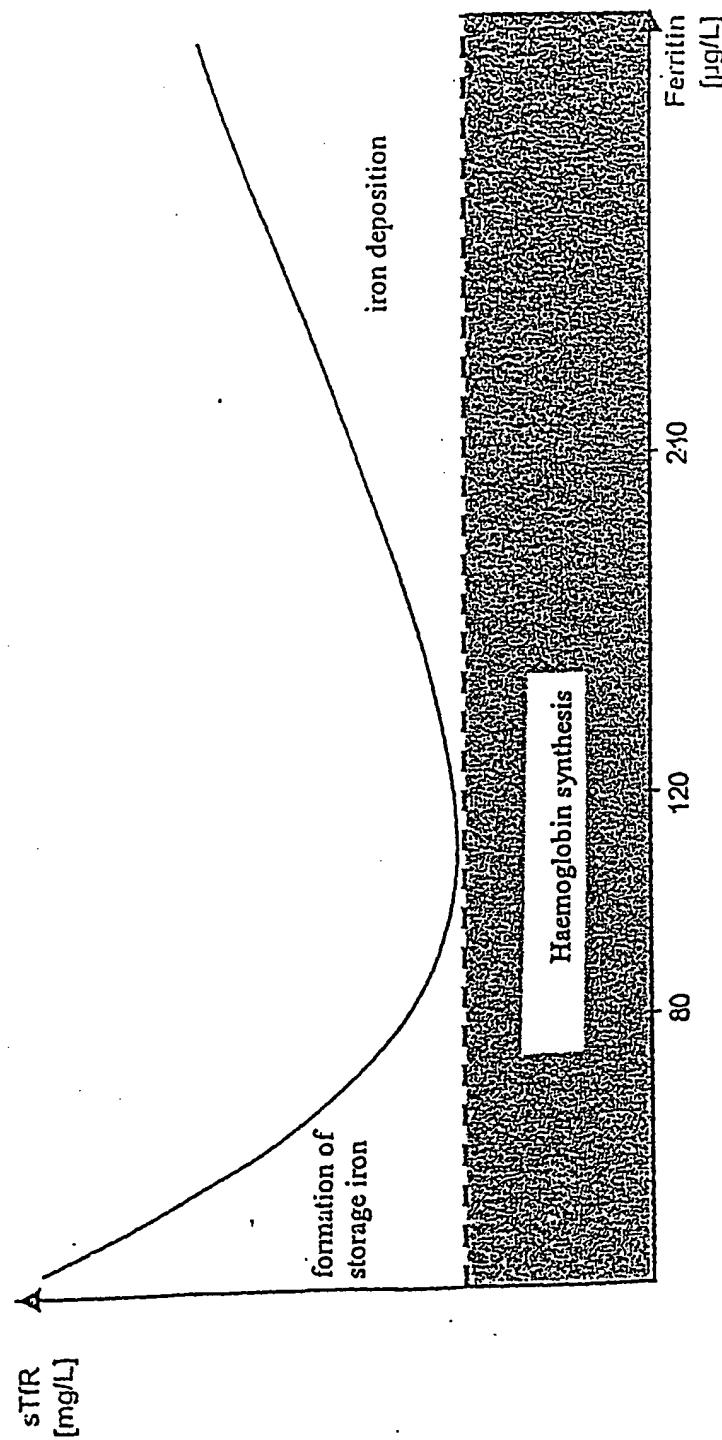


Figure 1

Fig. 2/4

Figure 2

<b>Differential diagnosis and monitoring of disorders of iron metabolism</b> Roche Diagnostics GmbH Sandhofer Strasse 116 D - 68305 Mannheim		<b>Sender (external)</b> Hospital _____ Ward _____ Telephone _____		<b>Patient:</b> Sample receive _____	
<b>Proteins:</b> sTfR (transferrin) Ferritin CRP		<b>Reference range:</b> sTfR (mg/L) Women (premenopausal): 1.9 - 4.4 mg/L Men: 2.2 - 5.0 mg/L Ferritin (µg/L) Women (premenopausal): 15 - 150 µg/L Women (premenopausal): 30 - 400 µg/L Men: 30 - 400 µg/L (consensus value): < 5 µg/L <b>Data Input</b>		<b>Haematology:</b> Haemoglobin (g/dL) Erythrocytes (10 <sup>12</sup> /µL) Haematocrit (%) MCV (fl) MCH (pg) CHr (pg)	
<b>Parenteral iron supply:</b> Hb, measured (g/L) Hb target value (g/L) Weight (kg) Iron dose (calculated) (g) <b>Data Input</b>					
Erythropoietin dose: (IU) <b>Data Input</b>					
Quadrant	Disorders of iron metabolism	sTfR / Log Ferritin	CRP concentration	MCH or Hb content of reticulocytes	Therapy
A	disorders of iron distribution (potential) further clarification required	<3.7 (women) <3.4 (men)	> 5 mg/L	MCH: normal: no acute deficiency of functional iron  reduced: deficiency of functional iron (except (thalassemia))  CHr: normal: normal functional iron status  reduced: acute deficiency of functional iron	—  Iron and erythropoietin supply  —  Iron and erythropoietin supply
B	storage iron overloading	<0.9 (women + men)	<5 mg/L	—	erythropoietin supply without iron substitution
C	normal storage iron status	0.9-3.7 (women) 0.9-3.4 (men)	<5 mg/L	—	—
D	iron deficiency	>3.7 (women) >3.4 (men)	> 5 mg/L	—	iron substitution
<b>Assessment:</b> _____					
<b>Recommended treatment:</b> _____					

Fig. 3/4

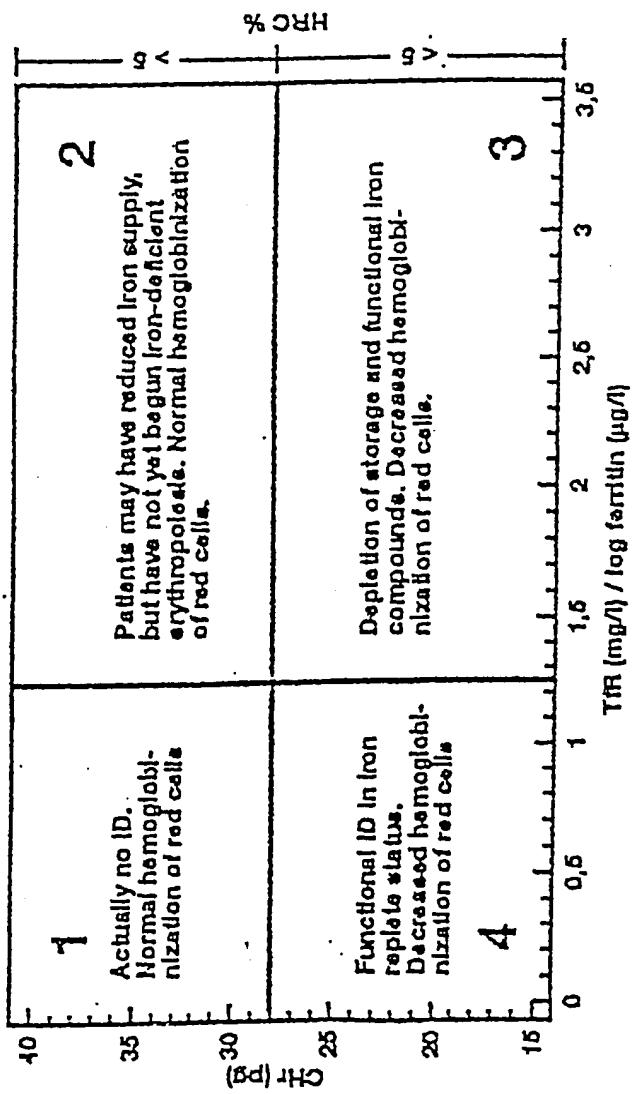
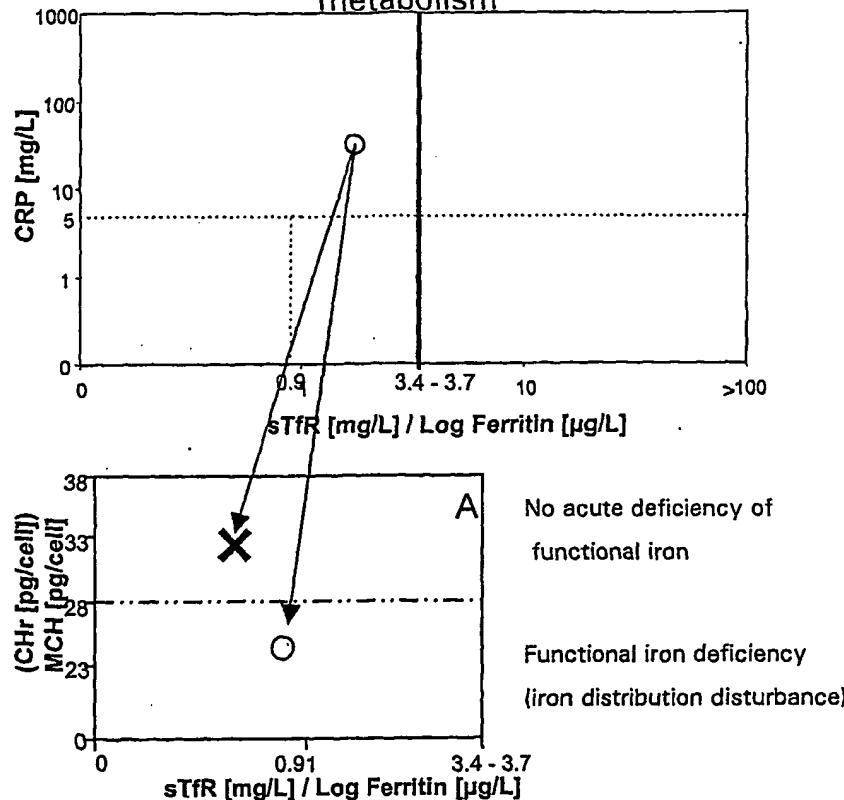


Figure 3

Fig. 4/4  
Differential Diagnosis and Monitoring of disorders of iron metabolism



Quadrant	disorders of iron metabolism	sTfR/log ferritin	CRP concentration	MCH or Hb content of reticulocytes	therapy
A	disorders of iron distribution (potential) further clarification required	<3.7 (women) <3.4 (men)	>5 mg/L	MCH: normal: no acute deficiency of functional iron reduced: deficiency of functional iron (except thalassemia) CH <sub>r</sub> : normal: normal functional iron status reduced: acute deficiency of functional iron	iron and erythropoietin supply iron and erythropoietin supply
B	storage iron overloading	<0.9 (women + men)	<5 mg/L	-	erythropoietin supply without iron substitution
C	normal storage iron status	0.9-3.7 (women) 0.9-3.4 (men)	<5 mg/L	-	-
D	iron deficiency	>3.7 (women) >3.4 (men)	>>5 mg/L	-	iron substitution

Figure 4